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Research Article

METHOD DEVELOPMENT AND ITS VALIDATION FOR QUANTITATIVE DETERMINATION OF BOSENTAN IN TABLET DOSAGE FORM BY RP-HPLC

*Shahul Hameed M, Jat R. K., Indulatha V. N.

Institute of pharmacy, Shri Jagdish Prasad Jhabarmal Tibrewala University, Vidyanagari, Jhunjhunu, Rajasthan, India-333001

ABSTRACT

Objectives of this research work were development and validation of HPLC methods of analysis for Bosentan in tablet dosage form from single components as per the current ICH & USP guidelines. HPLC methods also validated for the marketed Bosentan from single components. This developed procedure applied for regular analysis of these medicaments in pharmaceutical industry. The major scope of research is development of simple, accurate, reproducible & fast cost effective methods for new cephalosporins. The methods are validated with recovery studies using bulk drug of 80%, 120% & 40%. Specific method is confirmed by checking interference of excipients & assay method. The interday & intraday assay is also performed for checking robustness of the system. The minimum detection limit is checked by using formula $LOD = 3.3 \text{ sigma (Standard deviation)} / \text{Slop}$, where σ indicate standard deviation & S denotes slop of the regression straight line. The quantification limit is determined by using $LOQ = 10 \sigma \text{ (St\&. dev.)} / S$ that is minimum concentration of drug can be quantified. Linearity is found in the limit of Beer's law, straight line was constructed within the given range of the conc. of the drugs. So can develop & validate a new, reproducible, correct, & easy, less time consuming, cheap & eco-friendly method for daily analysis of drug in our general life.

Keywords: Bosentan, RP HPLC, method development and validation.

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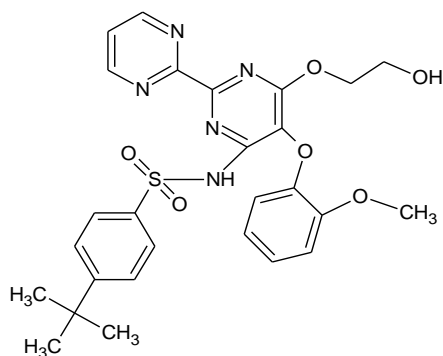
*Address for Correspondence

Shahul Hameed M, Institute of pharmacy, Shri Jagdish Prasad Jhabarmal Tibrewala University, Vidyanagari, Jhunjhunu, Rajasthan, India-333001, Email: shahulqa@gmail.com

INTRODUCTION

Bosentan is used to treat pulmonary hypertension by blocking the action of endothelin molecules that would otherwise promote narrowing of the blood vessels and lead to high blood pressure. Metabolism of Bosentan occurs mainly in the liver by the action of cytochrome P (CYP) 450 3A4 and 2C9, which produces three metabolites: the hydroxylated (hydroxy) metabolite, the demethylated (phenol) metabolite and the hydroxylated and demethylated (hydroxy-phenol) metabolite¹⁻⁴. Bosentan is chemically, 4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl)pyrimidin-4-yl]benzene-1 sulfonamide⁵. Various liquid chromatography techniques have been developed

for the determination of bosentan in biological fluids⁶⁻⁷ and tablet dosage forms⁸. Karnaker RT, *et al.* (2010) reported RPHPLC procedure for the development & validation of Bosentan present in tab. formulations. The mobile phase was selected on the hit & trial basis. The run time for mobile phase is 1 ml/minutes & gradient technique was used for continue elution of mobile phase according to volume time ratio⁸.



The method was developed for bulk drug & then applied for pharmaceutical formulations. The method is validated for recovery studies (40, 80, 120%), linearity, (preparation of standard curve), detection of limit, limit of quantification, stability studies on temperature basis, specificity of instruments. The method was applied for routine analysis of drugs¹⁰.

In the present study a simple, rapid, precise and accurate stability indicating liquid chromatographic method was developed for the determination of BST in in tablet dosage forms and validated as per ICH guidelines.

MATERIAL AND METHODS:

1. Analytical Method Development^{10,11}

1.1 Equipments

The following instruments & equipments were used during the development studies:

Table 1: List of Equipments

HPLC instrument	Waters 2695 separations module with 2487/2489 dual wavelength absorbance detector with Empower chromatographic software.
pH meter	Thermo-Orion star series
Analytical balance	Mettler Toledo
Ultrasonic bath	POWER SONIC420 “Aarkey”
Water purification System	Milli Q Gradient A 10 ,Elix A 10 “Millipore”

1.2 Reagents & Chemicals

The following reagents & chemicals were used during the validation studies:

Table 2: List of reagents

Sr. No.	Name of the material	Grade	Make
1	Potassium Dihydrogen ortho Phosphate	ExcelsaR	Fisher Scientific
2	Methanol	HPLC	S.D. Fine
3	Acetonitrile	HPLC	Rankem
4	Water	HPLC	Inhouse

1.3 Working Standard

The following Working standard was used during the develop studies:

Bosentan in Salt form: Potency (%) 98.8

1.4 Parameter Wise Method Development Plan

A. Molecular weight:

Molecular weight of Bosentan is 321.82gm & in salt form molecular weight is 419.7 gm.

B. Column selection: Non-polar columns

C. Detector selection: Bosentan is a UV active compound. Therefore, UV detector has been chosen for detection.

D. Selection of wavelength: λ_{\max} of Bosentan is 220nm.

E. Mobile phase selection: In mobile phase preparation following solvent & buffer has been taken:

a) Acetonitrile is used as it is having the UV-cut off 190nm & is commonly used solvent.

b) Phosphate buffer was selected as buffer: 1.36 gm potassium di-hydrogen phosphate in 1 L Milli Q Water (pH Value-4.4)

1.5 Method Development Trials

Table 3: Trial no. 1 Chromatographic conditions

Column	Inertsil ODS-3V (150*4.6) mm, 5 μ m
Flow rate	1.0ml/min
Detector wavelength	220nm
Injection volume	10 μ L
Sample temperature	25°C
Column temperature	25°C
Detector	UV
Diluent	Buffer:ACN (75:25)
Mobile phase B	Acetonitrile
Mobile phase A	Buffer
Run time	20 mins.

Table 7: Trial no. 5 Chromatographic conditions

Column	Ultron ES OVM-(150-4.6) mm, 5 μ m
Sample temperature	25°C
Injection volume	10 μ L
Flow rate	1.0ml/min
Detector wavelength	220nm
Column temperature	25°C
Detector	UV
Coposition	Buffer: ACN (90:10)
Mobile phase B	Acetonitrile
Mobile phase A	Buffer
Run time	20 mins.

Table 4: Trial no. 2 Chromatographic conditions

Column	Kromasil C 18 (150*4.6) mm, 5 μ m
Sample temperature	25°C
Injection volume	10 μ L
Flow rate	1.0ml/min
Detector wavelength	220nm
Column temperature	25°C
Detector	UV
Composition	Buffer: ACN (75:25)
Mobile phase B	Acetonitrile
Mobile phase A	Buffer
Run time	20 mins

Table 8: Trial no. 6 Chromatographic conditions

Column	Ultron ES OVM (150*4.6) mm, 5 μ m
Sample temperature	25°C
Injection volume	10 μ L
Flow rate	1.0ml/min
Detector wavelength	220nm
Column temperature	25°C
Detector	UV
Diluent	BUFFER: ACN (80:20)
Mobile phase B	Acetonitrile
Mobile phase A	BUFFER
Run time	20 mins

Table 5: Trial no. 3 Chromatographic conditions

Column	Zorbax C 8 (150*4.6) mm, 5 μ m
Sample temperature	25°C
Injection volume	10 μ L
Flow rate	1.0ml/min
Detector wavelength	220nm
Column temperature	25°C
Detector	UV
Diluent	BUFFER:ACN (75:25)
Mobile phase B	Acetonitrile
Mobile phase A	BUFFER
Run time	20 mins.

Table 9: Trial no. 7 Chromatographic conditions

Column	Ultron ES OVM (150*4.6) mm, 5 μ m
Sample temperature	25°C
Injection volume	10 μ L
Flow rate	1.2ml/min
Detector wavelength	220nm
Column temperature	25°C
Detector	UV
Diluent	BUFFER:ACN (80:20)
Mobile phase B	Acetonitrile
Mobile phase A	BUFFER
Run time	20 mins.

Table 6: Trial no. 4 Chromatographic conditions

Column	Ultron ES OVM (150*4.6) mm, 5 μ m
Sample temperature	25°C
Injection volume	10 μ L
Flow rate	1.0ml/min
Detector wavelength	220nm
Column temperature	25°C
Detector	UV
Diluent	BUFFER:ACN (60:40)
Mobile phase B	Acetonitrile
Mobile phase A	BUFFER
Run time	20 mins.

Table 10: Trial no. 8 Chromatographic conditions

Column	Ultron ES OVM (150*4.6) mm, 5 μ m
Sample temperature	25°C
Injection volume	10 μ L
Flow rate	1.2ml/min
Detector wavelength	220nm
Column temperature	25°C
Detector	UV
Diluent	BUFFER:ACN (80:20)
Mobile phase B	Acetonitrile
Mobile phase A	BUFFER
Run time	20 mins.

2. Analytical Method Validation

2.1 Objective: To demonstrate that Assay method of Bosentan by Reverse Phase-high performance liquid chromatography (RP-HPLC) is suitable for intended purpose.

2.2 Scope: This validation study is applicable for Assay method of Bosentan, which will be used to ensure the identity, quality & purity of active pharmaceutical ingredient.

2.3 Methodology

2.3.1 Preparation of mobile phase-A

Dissolved 1.36 gm of potassium dihydrogen phosphate to 1000 mL of HPLC grade water. Mixed well using a magnetic stirrer bar until completely mixed. The solution was filtered through a 0.45 µm nylon membrane filter & degassed.

2.3.2 Preparation of mobile phase-B: Acetonitrile (HPLC Grade)

2.3.3 Preparation of standard solution

Weighed accurately about 48 mg of Bosentan working/reference standard into 50 mL volumetric flask. Dissolve in 30 mL of Methanol & dilute to volume with methanol. Diluted 5 ml of this solution to 50 ml Methanol

2.3.4 Preparation of sample solution

5 Intact tablet of Bosentan taken into 500 ml volumetric flask than added 10 ml of water & sonicate till disintegrated. Added about 400 ml of methanol & sonicated for 45 minutes than made up volume with methanol. Added 5 ml of this solution in to 50 ml volumetric flask & make up volume with methanol.

3. System Precision¹²

3.1 Chromatographic conditions

Table 11: Chromatographic conditions

Column	Ultron ES OVM (150*4.6) mm,5µm
Sample temperature	25°C
Injection volume	10µL
Flow rate	1.2ml/min
Detector wavelength	220nm
Column temperature	25°C
Detector	UV
Diluent	BUFFER:ACN (80:20)
Mobile phase B	Acetonitrile
Mobile phase A	BUFFER
Run time	20 mins.

Conc. (µg/mL) =

$$\frac{\text{Wt. taken (mg)} \times \text{Volume of Stock Solution taken (mL)} \times \text{Potency of St\&ard (on as is basis)} \times (\text{Molecular Weight of Drug 'X'}) \times 1000}{100 \text{ mL} \times \text{Dilution (mL)} \times \text{Molecular Weight of Bosentan salt form} \times 100}$$

Acceptance criteria: Acceptance criteria for system suitability should pass. Correlation coefficient should not be less than 0.990. Linearity has passed as accepted criteria.

3.2 HPLC analysis

Purge injector, seal wash, needle wash, wet prime & equilibrate the HPLC system until a steady baseline obtained (at least for 30 minutes) After the system has equilibrated & delta psi of system is less than 50, inject blank (Single injection), standard solution (5 replicate injection) & sample solution (in duplicate) into the chromatographic system.

3.3 Acceptance criteria for system suitability

1. The percentage relative standard deviation (%RSD) of drug peak areas for six replicate

Injectations of working standard solution should not be more than 2%.

2. The tailing factor of standard peak should not be more than 2.0

3. The plate count of standard peak should be more than 2000.

4. Method Precision:

Methodology: 5 intact tablet of Bosentan taken into 500 ml volumetric flask than added 10 ml of water & sonicate till disintegrated. Added about 400 ml of methanol & sonicated for 45 minutes than made up volume with methanol. Added 5 ml of this solution in to 50 ml volumetric flask & make up volume with methanol.

The sample was prepared six times as per above methodology & injected in duplicate into the HPLC system.

5. Linearity

Prepared system suitability solution & diluted standard solution as per STP. Injected blank, system suitability solution & diluted standard solution as per injection sequence. Checked the acceptance criteria for system suitability.

5.1 Preparation of Linearity Stock Solution

Weighed 99.52 gm of working standard in 100 ml volumetric flask. Added 30 ml of methanol & sonicated to dissolve. Made up volume with methanol.

Prepared Linearity solution of 20%, 80%, 120%, 160%, 240% by dilution of Linearity Stock solution as below

6. Accuracy

Prepared system suitability solution & diluted standard solution as per STP. Injected blank, system suitability solution & diluted standard solution as per injection sequence. Checked the acceptance criteria for system suitability.

6.1 Methodology

Accuracy weighed placebo equivalent as given below mg of Bosentan tablets & about 148 mg of Bosentan in salt form in 500ml of volumetric flask & dissolved with methanol & made up volume with methanol. Further diluted 5 ml of above solution in to 50 ml diluents. Filtered with 0.45 μ filter & injected in to HPLC

For 30% Accuracy- About 148 mg

For 100% Accuracy- About 493 mg

For 200% Accuracy- About 985 mg

RESULTS AND DISCUSSION

In order to get the increased efficiency of the chromatographic system, the conditions of experiment such as column, column temp, pH and composition of mobile phase, and detection wavelength were optimized by changing one parameter at a time and keeping the other parameters constant. In RP HPLC method, the primary requirement for developing a method for analysis is that the using different solvents and buffers and columns to get better retention time and theoretical plates, and better cost effective and time saving method than the previously developed methods.

Method Development Trials

Trial no.-1

Trials Observations:

- Run time is longer
- Peak is not eluted.
- Base line is not good.

Corrections to next trial: Column need to be changed

Trial no.-2

Trials Observations:

- Run time is longer
- Peak is not eluted.
- Base line is not good.

Corrections to next trial: Column need to be changed

Trial no.-3

Trials Observations:

- Run time is longer
- Peak is not eluted.
- Base line is not good.

Corrections to next trial: Mobile Phase composition needs to be changed and Column need to be changed

Trial No.4

Trials Observations:

- Run time is longer
- Peak is not eluted.
- Base line is good.
- Peak might be eluting earlier in dead volume due to increase ratio of Acetonitrile

Corrections to next trial: Mobile Phase Composition needs to be changed

Trial no.-5

Trials Observations:

- Base line is good but the retention of the Drug in the column was too much.
- No Sharpness of the peaks.
- Run time is too longer.
- Peak is eluted too late
- Tailing is high
- .Plate count is low

Corrections to next trial: Mobile phase composition needs to be changed.

Trial No.6

Trials Observations:

- Run time is longer
- Peak shape is good
- Base line is good.
- Peak is eluted late.

Corrections to next trial: Flow rate need to be changed and Run time need to be changed

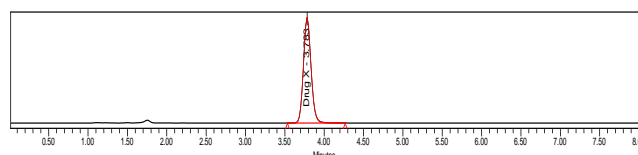
Trial No.7

Trials Observations:

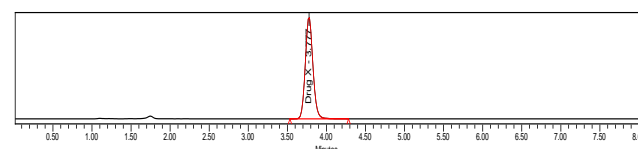
- Base line is good
- Sharpness of the peaks is fine
- Run time is optimum.
- Retention time is good (earlier)
- Tailing is low
- Plate count is high

Final Optimized Method

Related chromatogram: for Standard Solution



Related chromatogram: for Sample Solution



Analytical Method Validation

Mobile phase-A is prepared by Dissolving 1.36 gm of potassium di hydrogen phosphate to 1000 mL of HPLC grade water. Mixed well using a magnetic stirrer bar until completely mixed. The solution was filtered through a 0.45 µm nylon membrane filter & degassed. Mobile phase-B is prepared using Acetonitrile (HPLC Grade)

Standard solution is prepared by Weighing accurately about 48 mg of Bosentan working/reference standard into 50 mL volumetric flask. Dissolve in 30 mL of Methanol & dilute to volume with methanol. Diluted 5 ml of this solution to 50 ml Methanol

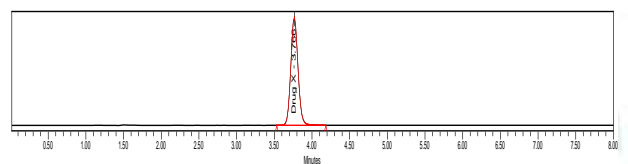
System Precision

Table 12: Chromatographic conditions

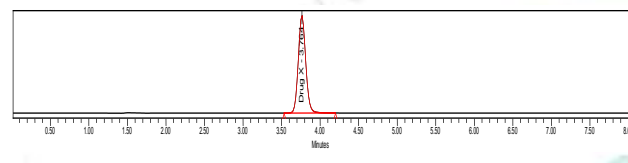
Column	Ultron ES OVM (150*4.6) mm,5µm
Sample temperature	25°C
Injection volume	10µL
Flow rate	1.2ml/min
Detector wavelength	220nm
Column temperature	25°C
Detector	UV
Diluent	BUFFER:ACN (80:20)
Mobile phase B	Acetonitrile
Mobile phase A	BUFFER
Run time	20 mins.

Chromatograms of Standard:

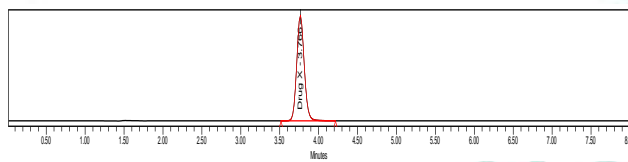
Injection-1



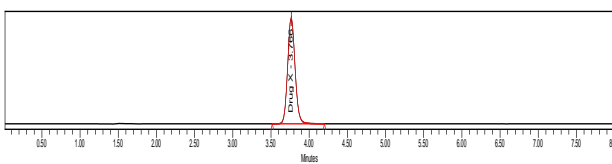
Injection-2



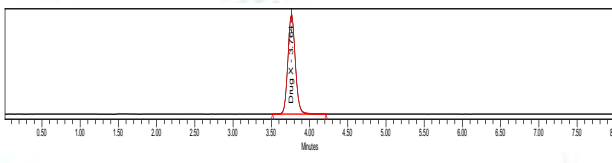
Injection-3



Injection-4



Injection-5



Injection-6

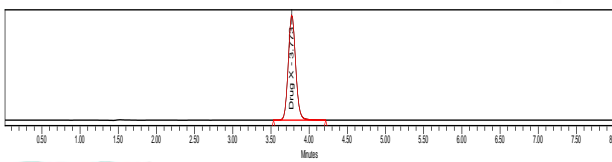


Table 13:

Standard Area Counts	
Injection	Area
1	1926300
2	1937992
3	1938435
4	1934998
5	1940483
6	1938834
Mean	1936174
S.D.	5156.3
% RSD	0.27

Using the chromatography data acquisition system, integrate the peaks of standard solution.

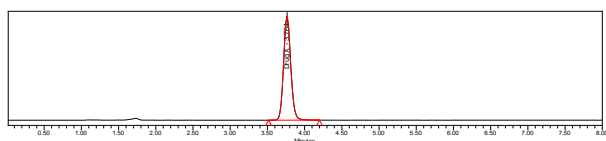
System suitability passes as per acceptance criteria.

Method Precision:

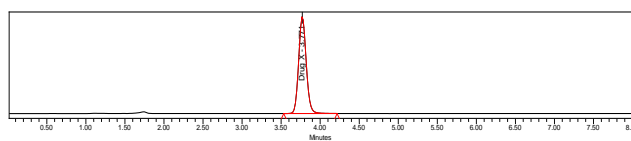
5 Intact tablet of Bosentan taken into 500 ml volumetric flask than added 10 ml of water & sonicate till disintegrated. Added about 400 ml of methanol & sonicated for 45 minutes than made up volume with methanol. Added 5 ml of this solution in to 50 ml volumetric flask & make up volume with methanol. The sample was prepared six times as per above methodology & injected in duplicate into the HPLC system.

Chromatograms of Method Precision

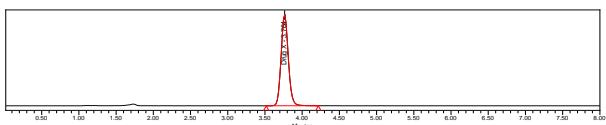
MP-1(Injection-1)



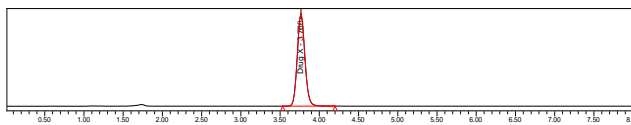
MP-4(Injection-1)



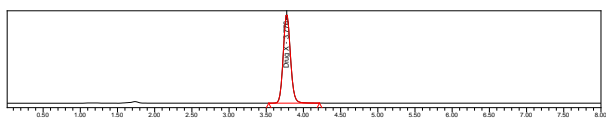
MP-1(Injection-2)



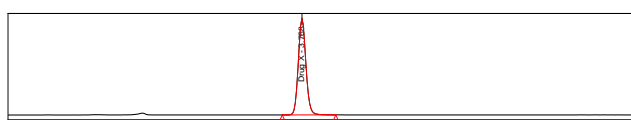
MP-4(Injection-2)



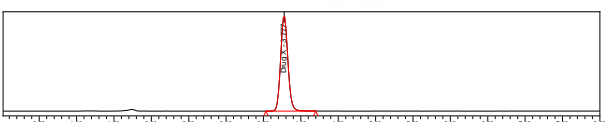
MP-2(Injection-1)



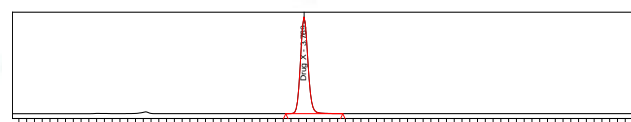
MP-5(Injection-1)



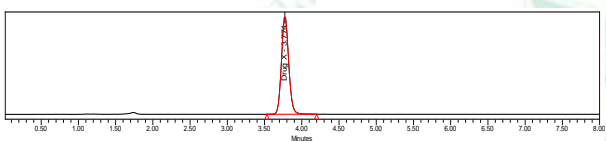
MP-2(Injection-2)



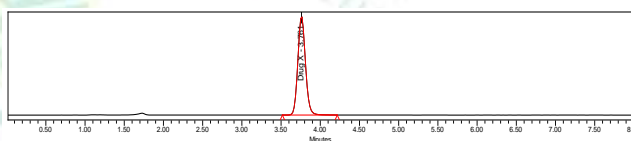
MP-5(Injection-2)



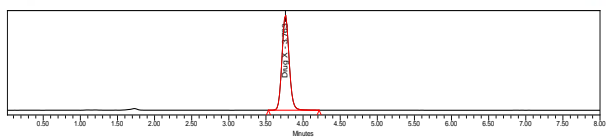
MP-3(Injection-1)



MP-6(Injection-1)



MP-3(Injection-2)



MP-6(Injection-2)

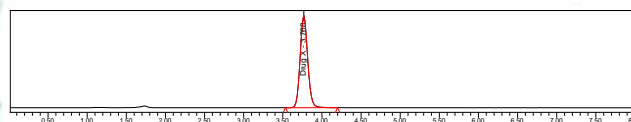


Table 14: Calculation of Method Precision

standard Area Counts	
Injection	Area
1	1926300
2	1937992
3	1938435
4	1934998
5	1940483
Mean	1935642
S.D.	5577.8
% RSD	0.29

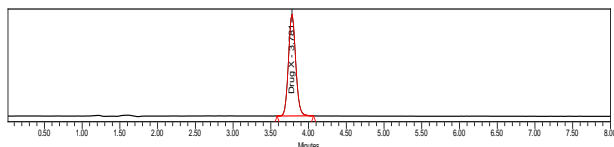
Conclusion: Method Precision has passed as per acceptance criteria.

Linearity

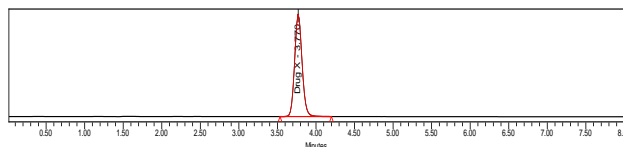
Prepared system suitability solution & diluted standard solution as per STP. Injected blank, system suitability solution & diluted standard solution as per injection sequence. Checked the acceptance criteria for system suitability.

Chromatogram of Linearity

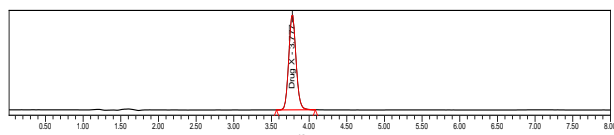
Linearity at 20 % (Injection-1)



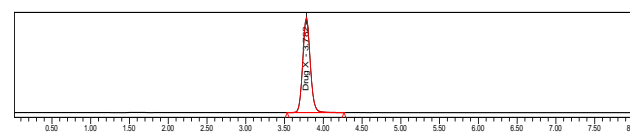
Linearity at 120 % (Injection-2)



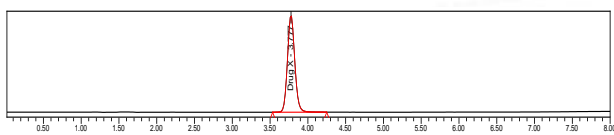
Linearity at 20 % (Injection-2)



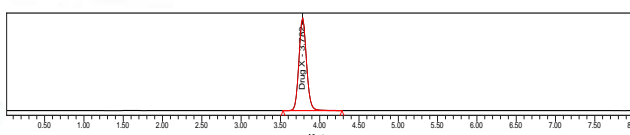
Linearity at 160 % (Injection-1)



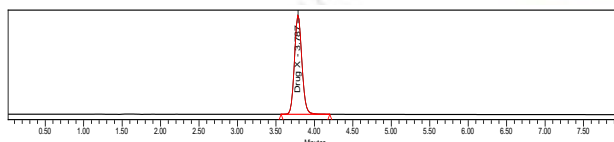
Linearity at 80 % (Injection-1)



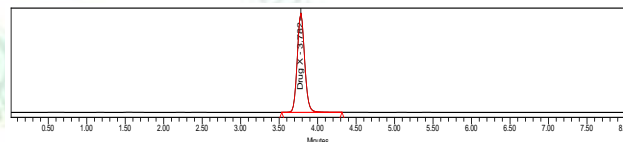
Linearity at 160 % (Injection-2)



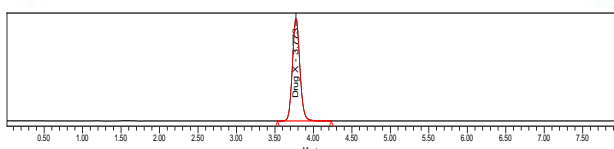
Linearity at 80 % (Injection-2)



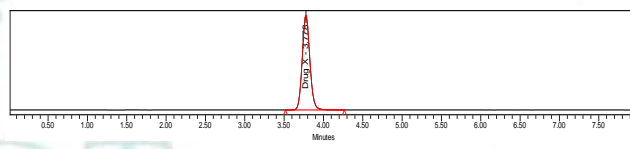
Linearity at 240 % (Injection-1)



Linearity at 120% (Injection-1)



Linearity at 240 % (Injection-2)



Preparation of Linearity Stock Solution

Weighed 99.52 gm of working standard in 100 ml volumetric flask. Added 30 ml of methanol & sonicated to dissolve. Made up volume with methanol. Prepared Linearity solution of 20%, 80%, 120%, 160%, 240% by dilution of Linearity Stock solution as below

Table 15: Preparation of Linearity Stock Solution

Sample ID	Linearity Stock Solution(mL)	Dilution (mL)	Linearity range (%)	Conc. (µg/mL)
Linearity - 1	2	100	20.1	15.08
Linearity - 2	4	50	80.4	60.32
Linearity - 3	6	50	120.6	90.47
Linearity - 4	8	50	160.8	120.63
Linearity - 5	6	25	241.3	180.95

Table 16: Calculation of Linearity

Standard Area Counts	
Injection	Area
1	1926300
2	1937992
3	1938435
4	1934998
5	1940483
Mean	1935642
S.D.	5577.8
% RSD	0.29

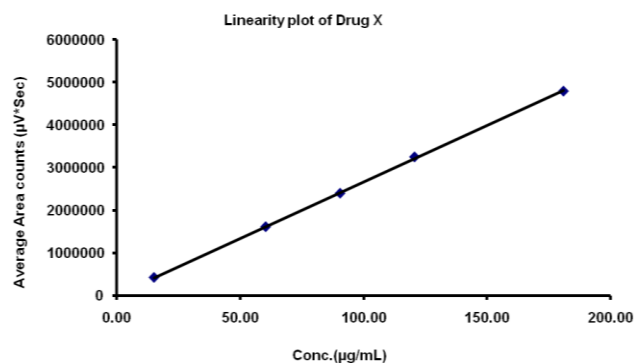


Figure 1: Linearity plot of Bosentan

Table 17: Calculation of Linearity

Molecular Wt of Bosentan: 321.82,

Molecular Wt of Bosentan salt form: 419.90,

Preparation of Linearity Stock solution: 99.52 gm to 100ml

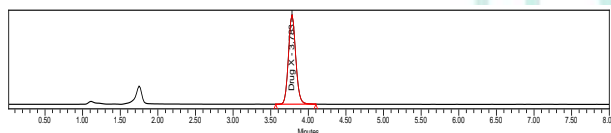
Linearity range (%)	Area Counts		Average area counts
	Injection - 1	Injection - 2	
20.1	416230	415094	415662
80.4	1610958	1598300	1604629
120.6	2391854	2389445	2390650
160.8	3237273	3244063	3240668
241.3	4790309	4777574	4783942
	Slope		26421.35
	Intercept		16977.76
Correlation Coefficient			0.9999

Acceptance criteria: Acceptance criteria for system suitability should pass. Correlation coefficient should not be less than 0.990. Linearity has passed as accepted criteria.

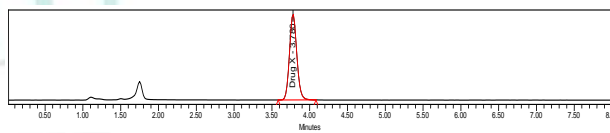
Accuracy

Prepared system suitability solution & diluted standard solution as per STP. Injected blank, system suitability solution & diluted standard solution as per injection sequence. Checked the acceptance criteria for system suitability.

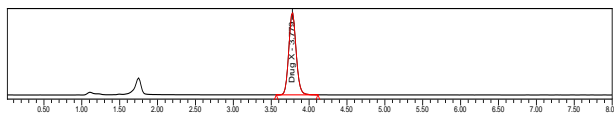
Accuracy at 30 % (Preparation-1, Injection-1)



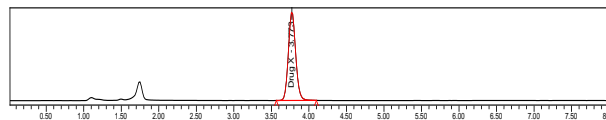
Accuracy at 30 % (Preparation-3, Injection-1)



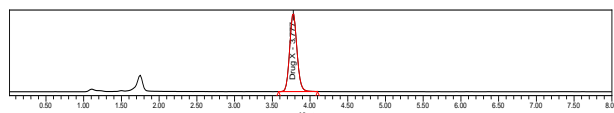
Accuracy at 30 % (Preparation-1, Injection-2)



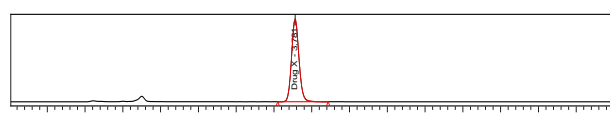
Accuracy at 30 % (Preparation-3, Injection-2)



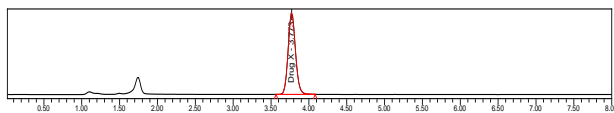
Accuracy at 30 % (Preparation-2, Injection-1)



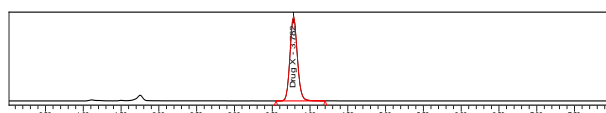
Accuracy at 100 % (Preparation-1, Injection-1)



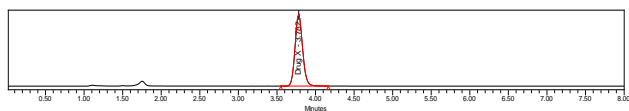
Accuracy at 30 % (Preparation-2, Injection-2)



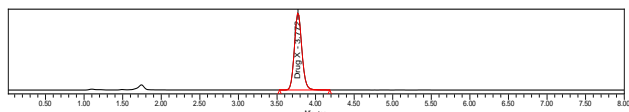
Accuracy at 100 % (Preparation-1, Injection-2)



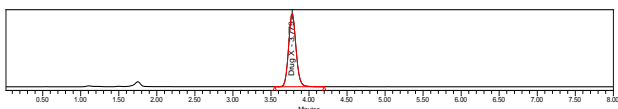
Accuracy at 100 %(Preparation-2, Injection-1)



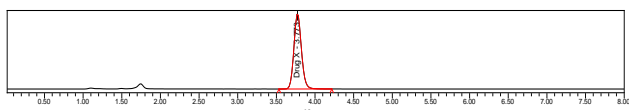
Accuracy at 100 %(Preparation-2, Injection-2)



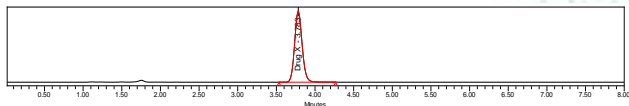
Accuracy at 100 %(Preparation-3, Injection-1)



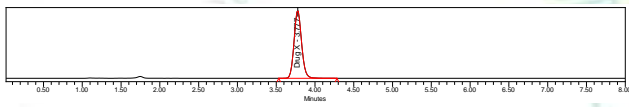
Accuracy at 100 %(Preparation-3, Injection-2)



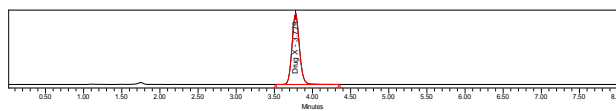
Accuracy at 200 %(Preparation-1, Injection-1)



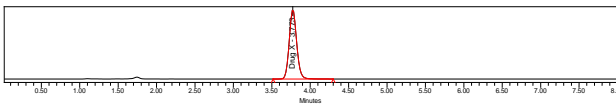
Accuracy at 200 %(Preparation-1, Injection-2)



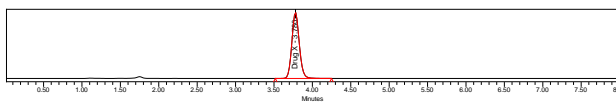
Accuracy at 200 %(Preparation-2, Injection-1)



Accuracy at 200 %(Preparation-2, Injection-2)



Accuracy at 200 %(Preparation-3, Injection-1)



Accuracy at 200 %(Preparation-3, Injection-2)

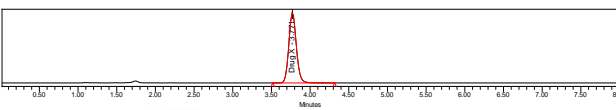


Table 18: Calculation of Accuracy

Standard Area Counts	
Injection	Area counts
1	1926300
2	1937992
3	1938435
4	1934998
5	1940483
Mean	1935642
S.D.	5577.8
% RSD	0.29

Table 19:

Sample ID	Accuracy Level	Weight taken (mg)	Actual Amount Added (mg)	Conc. (µg/mL)	Area Counts		
					Inj.1	Inj.2	Mean Area
Accuracy-30%-1	29.9	147.91	112.00	22.40	586163	586154	586159
Accuracy-30%-2		148.01	112.08	22.42	586244	586289	586267
Accuracy-30%-3		148.23	112.24	22.45	586348	586326	586337
Accuracy-100%-1	99.5	492.84	373.19	74.64	1944499	1944498	1944499
Accuracy-100%-2		492.92	373.25	74.65	1954631	1954335	1954483
Accuracy-100%-3		493.10	373.39	74.68	1964521	1965421	1964971
Accuracy-200%-1	198.9	984.98	745.85	149.17	3856895	3856785	3856840
Accuracy-200%-2		985.19	746.01	149.20	3885687	3886789	3886238
Accuracy-200%-3		985.46	746.21	149.24	3918568	3915678	3917123

Table 20: Accuracy recovery

Sample ID	Accuracy Level	Amount Recovered (mg)	% Recovery		
Accuracy-30%-1	29.9	112.13	100.12	Mean	100.04
Accuracy-30%-2		112.15	100.06	SD	0.097
Accuracy-30%-3		112.16	99.93	% RSD	0.10
Accuracy-100%-1	99.5	371.98	99.68	Mean	100.17
Accuracy-100%-2		373.89	100.17	SD	0.495
Accuracy-100%-3		375.89	100.67	% RSD	0.49
Accuracy-200%-1	198.9	737.80	98.92	Mean	99.66
Accuracy-200%-2		743.43	99.65	SD	0.750
Accuracy-200%-3		749.33	100.42	% RSD	0.75

Table 21: Compiled Recovery Data

Sample Name	Accuracy Level	% Recovery
Accuracy-30%-1	29.9	100.12
Accuracy-30%-2		100.06
Accuracy-30%-3		99.93
Accuracy-100%-1	99.5	99.68
Accuracy-100%-2		100.17
Accuracy-100%-3		100.67
Accuracy-200%-1	198.9	98.92
Accuracy-200%-2		99.65
Accuracy-200%-3		100.42
Overall Mean		99.96
Overall SD		0.361
Overall %RSD		0.36

CONCLUSION

An Analytical method for Assay of Bosentan Tablet was developed by Reverse phase High performance liquid chromatography by using different columns ,different mobile phase ratio & different chromatographic conditions & finally optimized using best chromatographic conditions.

The method was validated by the following parameters, system precision, method precision, accuracy, & linearity. All these parameters have passed as per acceptance criteria.

Finally in a concise & fruitful way, the developed method found to have several advantages over Traditional method:

- The method is cheap – Only 5 Tablets require at the place of 20 Tablets.
- The method is more accurate – In Direct intact method there is very less chances of contamination as compare to crush method.
- The method is easy to run – Developed method is easier as compare to crush method.
- The method consume Less time – Direct intact method has ability to save time of crushing, & shorter run time in chromatography.

There are five drugs that are analyzed in this research work. All drug have the same application as antihypertensive agents to relive the blood pressure. The drug are newer in this category. The drug bosentan is angiotensin II antagonist & latest in this category. The method for analysis is developed with high performance liquid chromatography. The method is simple, easy, accurate, sensitive, cost effective & rapid. The method is validated

according to international conference of harmonization for accuracy, precision, correctness, reproducibility, range, linearity, limit of detection, limit of quantification, robustness, ruggedness, & system suitability parameters. The method is then applied to marketed formulations. The method of analysis of bosentan can be applied for daily routine analysis of bulk drug bosentan & its marketed formulations.

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